Interleukin-2 induced immune effects in human immunodeficiency virus-infected patients receiving intermittent interleukin-2 immunotherapy

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To characterize the immunological effects of intermittent IL-2 therapy, which leads to selective increases in CD4+ T lymphocytes in HIV-infected patients, 11 patients underwent extensive immunological evaluation. While IL-2 induced changes in both CD4+ and CD8+ cell number acutely, only CD4+ cells showed sustained increases following discontinuation of IL-2. Transient increases in expression of the activation markers CD38 and HLA-DR were seen on both CD4+ and CD8+ cells, but CD25 (α chain of the IL-2 receptor) increased exclusively on CD4+ cells. This increase in CD25 expression was sustained for months following discontinuation of IL-2, and was seen in naive as well as memory cells. IL-2 induced cell proliferation, but tachyphylaxis to these proliferative effects developed after 1 week despite continued IL-2 administration. It thus appears that sustained CD25 expression selectively on CD4+ cells is a critical component of the immunological response to IL-2, and that intermittent administration of IL-2 is necessary to overcome the tachyphylaxis to IL-2-induced proliferation.

Key words: AIDS/HIV / Immunomodulator / Cytokine / T lymphocyte

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1 Introduction

The immunodeficiency of HIV infection is a complex interplay between the HIV-mediated destruction of CD4+ T cells and the capacity of the immune system to regenerate and maintain itself. Intermittent interleukin-2 (IL-2) therapy has been shown to shift the balance in many patients to favor CD4+ cell expansion. Controlled trials have shown that intermittent administration of IL-2 (originally called T cell growth factor [1]) at doses of 3×10^6–18×10^6 IU/day for 5 days by continuous intravenous or subcutaneous administration every 2 months can result in substantial CD4+ count increases, and that these increases can be sustained for over 5 years [2–5]. While daily administration of IL-2 leads to expansion of natural killer (NK) cells, it is the use of this cytokine in an intermittent fashion that leads to sustained increases in the CD4+ T cell pool [2, 6, 7]. This expansion is selective for CD4+ cells. CD8+ T cell and NK cell number are not affected.

To better understand the effects of IL-2 in HIV-infected patients, the current study was undertaken to examine in detail the immunological changes that occur during and after IL-2 therapy, and to attempt to delineate the mechanisms underlying the profound increases in CD4+ T lymphocyte count seen with this approach.

2 Results

Eleven male patients (mean age 35 years; range 31–42 years) enrolled in the study, throughout which they received anti-retroviral therapy (limited to zidovudine, didanosine, zalcitabine, or stavudine when the study was conducted) either alone (two patients) or in two drug combination regimens, and received three to six cycles of IL-2; three patients came off study after three cycles because their CD4+ counts did not demonstrate a sus-

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Abbreviation: BrdU: Bromodeoxyuridine

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tained increase, as previously reported [8]. Eight patients remain on study and continue to receive IL-2 periodically as needed to maintain CD4+ count increases at approximately double the baseline values (Fig. 1).

Table 1 summarizes immunological data prior to beginning therapy, after three cycles of IL-2 (approximately 6 months, for all 11 patients) and after 1 year (for the 8 patients who remained on study). These results, which describe the effects of 1 year of intermittent IL-2 therapy in this cohort, are similar to our previous observations [2–5]. Most noteworthy are the changes in CD4+ cells, with a marked increase in both CD4+ number and percent. This was associated with a profound increase in CD4+/CD25+ cells, from 11% to 43% of total CD4+ cells (2.3% and 16.5% of total lymphocytes, respectively). There was an increase in both naive (CD45RO−) and memory (CD45RO+) CD4+ cells, with an increase in the naive:memory ratio during the study. While there was a drop in the CD8+ percent, CD8+ cell number remained unchanged. Monocytes (CD14+), B cells (CD19+) and NK cells (CD16+/CD56+) remained largely unchanged during the year.

Changes in CD4+ and CD8+ number and percent during the first cycle of IL-2 are shown in Fig. 2A. During this cycle, six patients received the scheduled 5 days of IL-2, and the remaining five discontinued therapy during the 5th day due to side effects, primarily constitutional

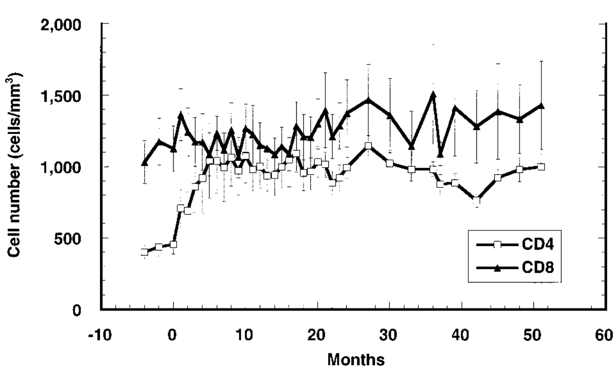

Fig. 1. Mean CD4+ and CD8+ cell number for eight responding patients receiving intermittent IL-2, initially every 2 months, and subsequently less frequently as needed to maintain the CD4+ count increase. In the 44 months after the initial year of study, one patient maintained his CD4+ count increase without additional IL-2. The remaining seven patients received an average of seven cycles (range 2–10); during this time, the mean interval between cycles per patient ranged from 3.5 to 16 months (mean 7.3 months), and the mean dose per cycle was 32×10^6 IU. Month 0 represents the time when the first IL-2 cycle was administered. Error bars indicate the standard error for each value.

symptoms. Immediately after starting a cycle of IL-2, a rapid decline in the absolute number of both CD4+ and CD8+ cells in the periphery was seen, with a gradual increase during the last 2 days of the infusion. Following discontinuation of IL-2, there was an immediate and marked increase in both cell populations, which gradually returned towards baseline over the following 2 months. However, whereas the CD8+ cell number returned to baseline, and did not increase following subsequent cycles, the CD4+ count remained above baseline values 2 months later. For the group as a whole, the CD4+ count was 39% above baseline (543 cells/mm^3 *vs*. 390 cells/mm^3 at baseline, p=0.057) immediately before beginning cycle 2. The CD4+ percent also remained above baseline (25% *vs*. 21%. p=0.002).

IL-2 therapy was associated not only with changes in the number of lymphocytes, but also with changes in the expression of several markers of activation, including CD25 (α chain of the IL-2 receptor), HLA-DR, and CD38 (Fig. 2B, C). At the end of the IL-2 infusion, and especially during the subsequent 5 days, there was an increase in the number (baseline, 42 cells/mm^3 increasing to 613 cells/mm^3 at day 6) as well as percent (baseline, 2% increasing to 11% at day 6) of CD4+ cells expressing CD25; CD25 expression on CD4+ cells remained substantially above baseline values until the next IL-2 cycle [120 cells/mm^3 (p=0.031), 6% (p=0.004)] (Fig. 2B). In contrast, CD8+ cells did not show a similar increase in percent CD25 expression during or after an IL-2 cycle, although the absolute number of cells expressing CD25 did increase transiently after IL-2 was discontinued (Fig. 2C). It is likely that this differential expression of CD25 plays a role in the selective increase induced by intermittent IL-2 therapy in CD4+ compared to CD8+ T cells.

Similarly, the number and percent of CD4+ cells expressing HLA DR and CD38 also increased during and immediately after a cycle of IL-2, but unlike for CD25, the values returned to near baseline values by the next IL-2 cycle (Fig. 2B). CD8+ cells also showed a marked increase in both number and percent of cells expressing these markers, but these values had also returned to baseline by the next cycle (Fig. 2C).

CD4+/CD45RO+ isoform expression was examined to determine the impact of IL-2 on naive (CD45RO-) and memory (CD45RO+) cells (Fig. 2D). During the first cycle, there was an initial increase in the ratio of naive to memory cells, presumably resulting from a greater clearance of CD4+/CD45RO+ cells from the periphery. By day 4, however, the ratio had declined below baseline levels as CD45RO+ cells began to return to the periphery. Immediately following discontinuation of IL-2, the CD4+ cells

Table 1. Comparison of immunological parameters prior to and after 6 or 12 months of IL-2[a]

Parameter	Month 6 Comparison (n=11)			Month 12 Comparison (n=8)			HIV-negative Controls[b]
	Pre-IL-2	Post 3 cycles	p value	Pre-IL-2	Post 6 cycles	p value	
CD4 %	20.1 (±2.1)	32 (±4.9)	0.003	21.8 (±2.4)	38.2 (±4.0)	<0.001	47.5 (±0.5)
CD4 no.	371 (±51)	803 (±208)	0.026	427 (±59)	991 (±148)	0.001	903 (±20)
CD4/CD25 %	2.3 (±0.3)	10.8 (±2.4)	0.004	2.3 (±0.3)	16.5 (±3.2)	0.004	15 (±0.3)
CD4/CD25 no.	40 (±4)	269 (±79)	0.013	43 (±5)	412 (±75)	0.002	238 (±8)
CD4/DR %	4.4 (±0.5)	4.1 (±0.5)	NS[c]	4.0 (±0.3)	3.7 (±0.4)	NS	4.1 (±0.1)
CD4/DR no.	77 (±8)	84 (±11)	NS	79 (±9)	97 (±18)	NS	76 (±2)
CD4/CD38 %	13.2 (±1.2)	15.2 (±1.6)	0.02	13.8 (±1.4)	16.6 (±1.3)	NS	29.1 (±0.5)
CD4/CD38 no.	236 (±25)	338 (±63)	0.033	263 (±30)	418 (±48)	0.001	554 (±15)
CD4/CD45RA %	6.7 (±1.2)	14.4 (±3.6)	0.012	7.6 (±1.4)	16.3 (±3.2)	0.005	18.1 (±0.5)
CD4/CD45RA no.	122 (±27)	383 (±138)	0.048	148 (±33)	444 (±122)	0.019	349 (±12)
CD4/CD45RO %	14 (±1.1)	17 (±1.8)	0.004	14.4 (±1.3)	21 (±3.0)	0.028	29.1 (±0.4)
CD4/CD45RO no.	251 (±29)	400 (±78)	0.02	282 (±234)	523 (±66)	0.001	550 (±13)
CD8 %	58.7 (±2.8)	48.3 (±4.4)	0.003	55.8 (±3.2)	45.9 (±3.7)	0.001	24.7 (±0.5)
CD8 no.	1099 (±125)	1058 (±144)	NS	1115 (±152)	1187 (±179)	NS	463 (±12)
CD8/CD25 %	0.6 (±0.1)	1.6 (±0.2)	0.001	0.5 (±0.1)	2.9 (±0.6)	0.006	1.5 (±0.1)
CD8/CD25 no.	12 (±2)	34 (±5)	0.001	11 (±3)	75 (±20)	0.009	27 (±1)
CD8/DR %	23.4 (±3.5)	16.5 (±2.5)	0.006	23.3 (±3.1)	19 (±3.7)	0.032	4.9 (±0.2)
CD8/DR no.	445 (±82)	353 (±77)	NS	488 (±105)	517 (±141)	NS	92 (±4)
CD8/CD38 %	44.4 (±3.4)	34.4 (±4.3)	0.001	39.8 (±3.3)	30.6 (±3.8)	<0.001	12.5 (±0.3)
CD8/CD38 no.	807 (±88)	706 (±100)	NS	773 (±98)	781 (±136)	NS	235 (±7)
CD14 no.	351 (±38)	295 (±24)	NS	351 (±52)	302 (±22)	NS	406 (±10)
CD19 no.	211 (±51)	211 (±46)	NS	239 (±68)	236 (±46)	NS	245 (±8)
CD16/CD56 no.	120 (±15)	116 (±19)	NS	131 (±18)	121 (±22)	NS	229 (±7)

[a] Results represent the mean (±SE) of 3 pre-IL-2 values and 2 post IL-2 values. Values represent number of cells/mm^3 (no.) or percent of total lymphocytes (%).

[b] Mean (±SE) values for 229 to 238 HIV negative adults

[c] NS, not significant

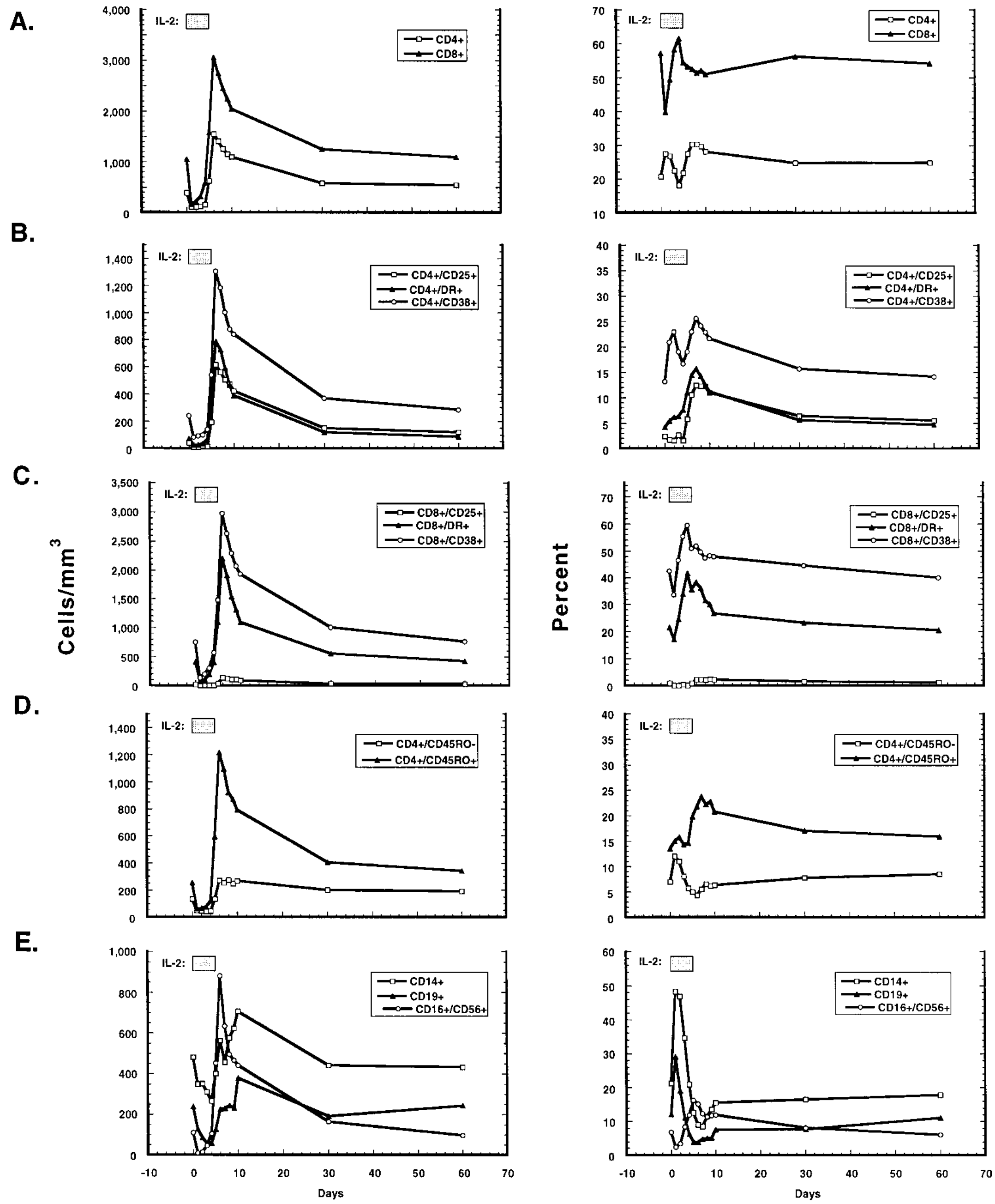

Fig. 2. Changes in lymphocyte subpopulations during and immediately after the first cycle of IL-2. Values represent the mean for all 11 patients at each time point. The bar indicates the 5 days during which IL-2 was administered by continuous infusion. (A) $CD4^+$ and $CD8^+$ cell number (left) and percent (right); (B) $CD4^+$ and (C) $CD8^+$ cell number (left) and percent (right) that were also positive for the activation markers CD25, HLA-DR, and CD38. (D) $CD4^+$ naive ($CD45RO^-$) and memory ($CD45RO^+$) cell number (left) and percent (right). (E) Monocytes ($CD14^+$), B cells ($CD19^+$), and NK cells ($CD16^+/CD56^+$) cell number (left) and percent (right). Percentages represent the percent of total lymphocytes, except for $CD14^+$, which represents the percent of lymphocytes plus monocytes.

appearing in the periphery were primarily $CD45RO^+$. Both populations gradually declined over the next 2 months, but remained above baseline values immediately prior to the next IL-2 cycle. Following 1 year of IL-2 therapy there was an increase in both naive and memory $CD4^+$ T cells with a preferential increase in the naive pool, such that the ratio of naive to memory $CD4^+$ T cells increased from 0.53 to 0.78.

During the 5 days of IL-2 administration, monocytes ($CD14^+$), B cells ($CD19^+$), and NK cells ($CD16/CD56^+$) all declined in number. The decline in monocytes and B cells was relatively less than for NK cells (Fig. 2E). Following discontinuation of IL-2, monocytes and B cells rapidly returned to baseline levels, while NK cells showed an increase similar to that seen with T cells, before returning to baseline.

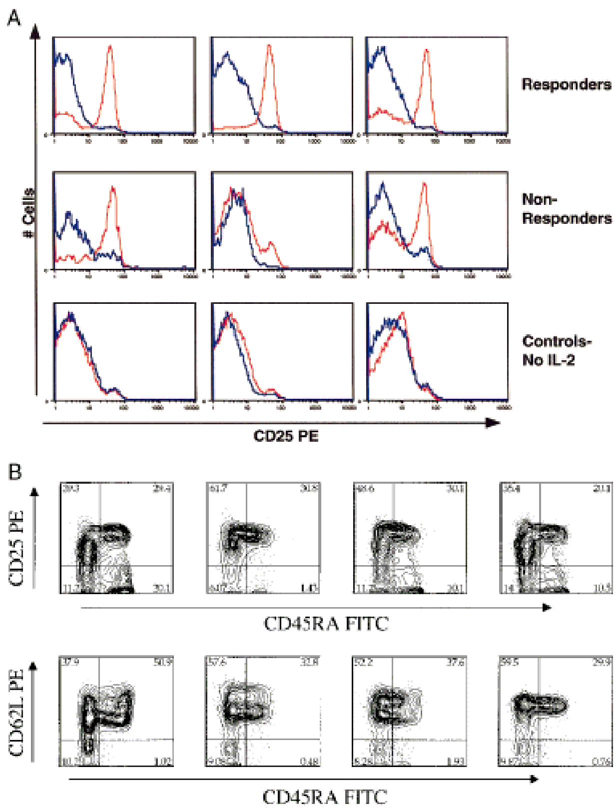

Fig. 3. Expression of CD25 by naive $CD4^+$ cells following IL-2 therapy. (A) Flow cytometry histograms are shown for six patients in the current study: three responders to IL-2 and the three non-responders, together with three controls who were randomized to the no IL-2 arm in a randomized IL-2 study [3]. Cells were obtained at study enrollment (blue line) and 5 months (non-responders) or 11 months (responders and controls) (red line) later. Gating was on naive $CD4^+$/$CD27^+$/$CD45RO^-$ lymphocytes. Number of cells is shown along the y axis, and intensity of staining with CD25 along the × axis. Frozen PBMC were thawed and processed simultaneously for each patient. Responders demonstrated a substantial increase in CD25 expression by naive cells that is seen to a lesser extent in non-responders, and is not seen in the controls. The results for the responders is representative of results in the other responders; similar results were seen at 5 months. (B) Flow cytometry profiles for four patients participating in ongoing IL-2 trials. The most recent IL-2 cycle was administered 48, 15, 4 and 1 month previously, respectively. Gating was on $CD3^+$/$CD4^+$ cells. The top set of flow profiles demonstrates that the majority (58–95%) of $CD4^+$/$CD45RA^+$ cells also express CD25 in all four patients. The bottom panels demonstrate that greater than 95 % of the $CD45RA^+$ cells are also $CD62L^+$ naive cells.

Given that IL-2 therapy induced a long-term increase in both naive $CD4^+$ cells and $CD4^+$/$CD25^+$ cells, expression of CD25 by naive cells was examined using frozen cells from the 11 patients as well as using fresh cells from 4 additional patients with $CD4^+$ count increases in response to IL-2 therapy administered as part of other ongoing IL-2 trials at the NIH. As shown in Fig. 3, there was marked up-regulation of CD25 expression by naive CD4 cells in both populations.

One of the most potent activities of IL-2 is the induction of T cell proliferation. To assess the impact of IL-2 on lymphocyte proliferation *in vivo*, levels of [^{3}H]thymidine incorporation (an indicator of DNA synthesis) were measured following a 4-h *in vitro* pulse of freshly isolated, unstimulated peripheral blood mononuclear cells (PBMC). The level of incorporation of [^{3}H]thymidine correlated well with the proportion of PBMC in S-phase as determined by cell cycle analysis using propidium iodide (r=0.97, p<0.001; Fig. 4A), and thus is a reliable marker of cell proliferation *in vivo*. During a 21-day infusion of IL-2 into patients receiving zidovudine alone as anti-retroviral therapy, levels of [^{3}H]thymidine incorporation peaked at 7 days in a dose-dependent fashion, with levels returning to baseline by 14–21 days (Fig. 4B). This transient increase in proliferation was seen despite the fact that the rate of IL-2 administration was constant over the 21-day period. $CD4^+$ counts similarly showed a transient increase that also peaked at day 7 in the highest dose group (Fig. 4B).

To examine proliferation in specific subpopulations, spontaneous *ex vivo* incorporation of bromodeoxyuridine (BrdU) by $CD4^+$ and $CD8^+$ cells during a 4-h incubation with fresh, unstimulated PBMC was examined prior to and 4–6 days after the start of an IL-2 cycle in 18 patients participating in ongoing IL-2 trials, all of whom were receiving combination anti-retroviral regimens that included a protease inhibitor. Both populations of cells showed a significant increase in BrdU incorporation in the setting of IL-2 therapy, with the mean incorporation increasing 14.5-fold for $CD4^+$ cells (0.16 to 2.32%, p<0.001) and 27-fold for $CD8^+$ cells (0.07 to 1.90%, p=0.005) (Fig. 4C).

To determine if the apparent tachyphylaxis to IL-2-induced proliferation seen during the 21-day infusions was at the level of the cell, cells obtained at day 21 from patients receiving IL-2 for 21 days were incubated

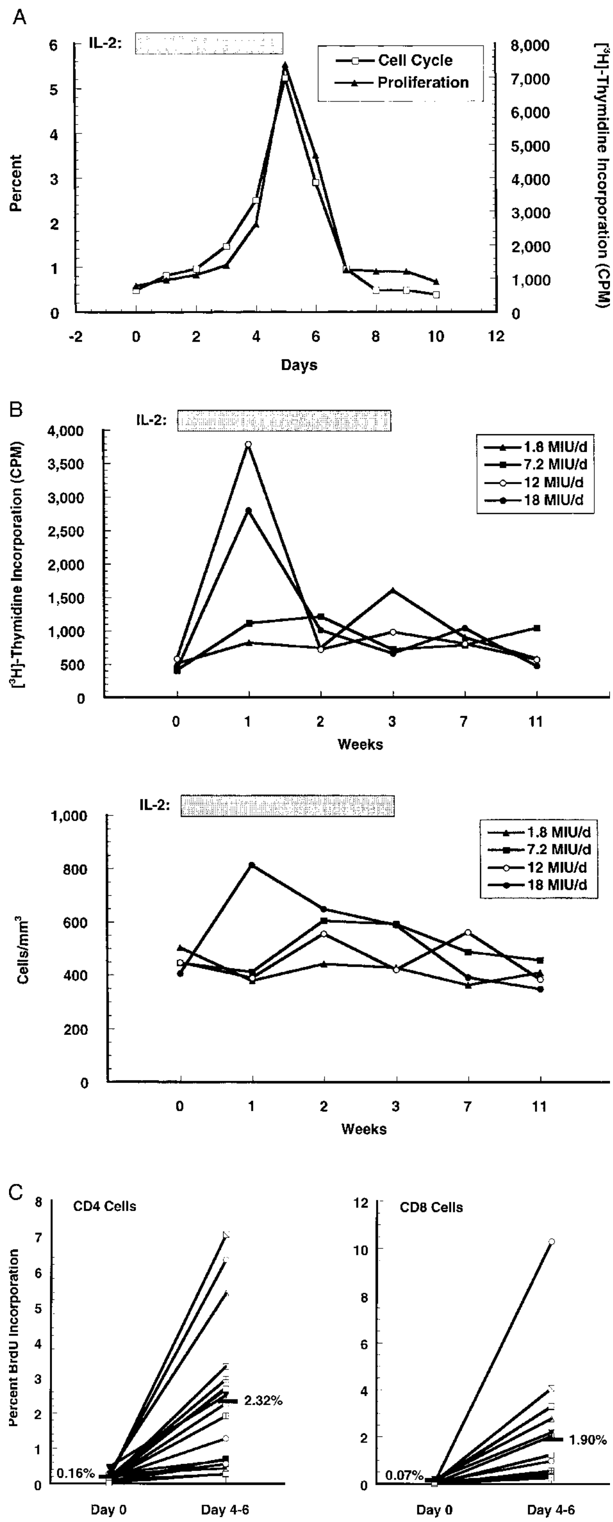

Fig. 4. (A) Proliferation of peripheral blood mononuclear cells as measured simultaneously by propidium iodide staining, to determine the percent of cells in S-phase (cell cycle), and [^{3}H]thymidine incorporation (proliferation) for six patients receiving a continuous infusion of IL-2. The bar indicates the period during which IL-2 was administered. (B) Mean spontaneous proliferation (top) and CD4$^+$ cell number (bottom) during 21-day IL-2 infusions. Each line plots the mean values for 3–5 patients per dosing group. Doses of 1.8×10^6–18×10^6 IU/day were administered by continuous infusion. There is a dose-dependent increase in both spontaneous proliferation, as measured by [^{3}H]thymidine incorporation, and CD4$^+$ counts, that peaks at week 1 and then declines despite continued administration of a constant dose of IL-2. Bar indicates the period during which IL-2 was administered. (C) IL-2 induced changes in spontaneous proliferation of CD4$^+$ cells (left panel) and CD8 cells (right panel), as measured by *ex vivo* BrdU incorporation. Whole blood samples obtained from 18 patients prior to and 4–6 days after starting an IL-2 cycle were incubated for 4 h with 10 μM BrdU, following which samples were processed for flow cytometry. Each line represents results for a single patient, with the mean value for the group at each time-point indicated by the bar and percentage value. There was a mean 14.5- and 27-fold increase in BrdU incorporation for CD4$^+$ cells ($p<0.001$) and CD8$^+$ cells ($p=0.005$), respectively, after 4–6 days of IL-2 therapy.

with IL-2 for 24–48 h *in vitro*. As shown in Fig. 5A, these cells were capable of responding to IL-2 *in vitro*. To determine if tachyphylaxis was due to alterations in the pharmacodynamics of IL-2, serial measurements were made of serum levels of IL-2 and the α chain of the IL-2 receptor (soluble CD25) during a 5-day cycle. As shown in Fig. 5B, serum levels of IL-2 peaked at around 24–48 h following the initiation of the infusion, and then declined from a peak of 93 U/ml to 21 U/ml at day 5, despite a constant rate of infusion of IL-2. This was seen in association with an increase in serum levels of CD25. Following the discontinuation of IL-2, these parameters returned to baseline and could again be perturbed 8 weeks later with the next 5-day cycle of IL-2.

3 Discussion

Through a detailed analysis of the immunological changes associated with IL-2 therapy, this study has provided insights into the mechanisms that may be responsible for the CD4$^+$ count increases that are seen with intermittent IL-2 therapy [2–5]. IL-2 induces proliferation of lymphocytes, as demonstrated by increased

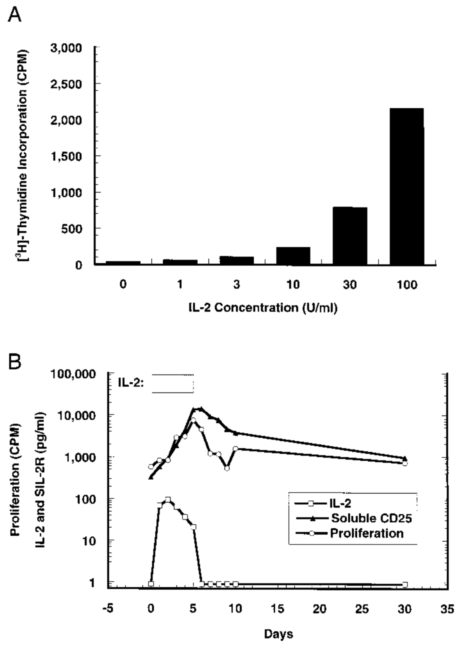

Fig. 5. (A) *In vitro* IL-2-induced proliferation, as measured by [^{3}H]thymidine incorporation, using cells obtained at day 21 of a 21-day cycle of IL-2. Frozen cells were thawed and incubated for 48 h with IL-2 at the concentration indicated at the bottom. Results represent the mean for five patients, three of whom received 12×10^6 IU/day, and two that received 18×10^6 IU/day. There was a dose-dependent increase in proliferation, indicating that despite the recent *in vivo* exposure to IL-2, there was no inherent tachyphylaxis of these cells to the proliferative effects of IL-2. (B) Temporal relationship between proliferation of peripheral blood mononuclear cells as measured by [^{3}H]thymidine incorporation (proliferation) and changes in serum levels of IL-2 and soluble CD25 (alpha chain of the IL-2 receptor). As the serum IL-2 receptor levels increase, there is a concomitant decrease in IL-2 levels despite the continued administration of a constant dose of IL-2.

spontaneous blast transformation, cell cycle analysis, and BrdU incorporation. Thus, the simplest interpretation of the observed immunological changes is that IL-2 is acting as an *in vivo* T cell growth factor [9].

Acutely, IL-2 affects both $CD4^+$ and $CD8^+$ cells, inducing not only proliferation, but also expression of activation markers such as HLA-DR and CD38, as well as changes in trafficking, as evidenced by the rapid fluxes of both populations during and immediately after discontinuation of IL-2. However, long-term effects on cell number are limited to the $CD4^+$ population [3, 5], and thus the proliferative effects alone cannot explain the sustained increase in $CD4^+$ cell number. This specificity may be a result of the differential induction by IL-2 of CD25, which as shown in this study is limited largely to $CD4^+$ cells, and to the continued expression of CD25 by $CD4^+$ cells, including naive $CD4^+$ cells, long after IL-2 has been discontinued. This ongoing expression can potentially maintain the $CD4^+$ increases through a number of mechanisms. CD25 expression may result in decreased rates of apoptosis and consequent prolonged survival in response to endogenously produced IL-2 [10]. Alternatively, cells that are expressing CD25 may be induced to proliferate by low levels of endogenously produced IL-2 or may have increased responsiveness to subsequent cycles of exogenously administered IL-2, since lower doses of IL-2 administered during subsequent cycles as maintenance therapy are often sufficient to maintain IL-2 receptor expression and $CD4^+$ cell expansion. However, preliminary *in vitro* studies examining the proliferative responses of these cells after 1 year of intermittent IL-2 therapy suggest that $CD4^+/CD25^+$ cells are less, not more, responsive to IL-2, suggesting that increased proliferation is not the driving mechanism to maintenance of these cells (data not shown). Similarly, a recent study has shown that decreased apoptosis, together with increased differentiation from bone-marrow progenitors, rather than increased proliferation of mature cells, is the mechanism accounting for the increase in NK cells that occurs with daily low-dose IL-2 therapy [11].

An early phase of this study demonstrated tachyphylaxis to the proliferative effects of IL-2 during 21 days of continuous infusion. Proliferation as measured by spontaneous blast transformation peaked at 1 week and then returned to baseline levels despite continued administration of IL-2 (Fig. 4B). However, the cells remained responsive to IL-2 *in vitro*, demonstrating that the cells were not inherently resistant to the effects of IL-2. In a more detailed study, declines in IL-2 induced proliferation were seen at days 6–8 of ongoing IL-2 therapy [11 a]. These data suggest that *in vivo* a rest period is necessary before stimulated cells will regain susceptibility to the proliferative effects of IL-2. Subsequent studies limited the duration of IL-2 administration to 5 days based in part on these data. During 5-day infusions at a constant rate, there is a substantial decline in serum IL-2 levels. An up-regulation of membrane bound CD25, as documented by flow cytometry, may account for this increase in IL-2 metabolism via increased receptor-mediated endocytosis, resulting in a decline in IL-2 levels and the observed tachyphylaxis. In support of this is the previously reported inverse correlation between serum levels of IL-2 and serum levels of soluble CD25, which has presumably been shed by activated cells expressing this receptor [12].

IL-2 therapy resulted in long-term expansion of both $CD4^+/CD45RO^+$ (memory) and $CD4^+/CD45RO^-$ ($CD45RA^+$, naive) cells, with a preferential expansion of naive cells, as we and others have previously reported [13, 14]. During and immediately after a cycle, however, the bulk of cells take on the activated memory phenotype ($CD45RO^+$), which is then rapidly lost, either by recirculation out of the periphery, by reversion from the $CD45RO^+$ to $CD45RO^-$ phenotype, or by apoptosis. It is noteworthy that, *in vitro*, $CD4^+$/$CD45RA^+$ cells can be stimulated to grow and maintain the naive phenotype in a milieu of cytokines that includes IL-2, TNF-α and IL-6, which is the cytokine milieu that occurs *in vivo* in patients receiving IL-2 [15]. The naive $CD4^+$ cells that have expanded *in vivo* during IL-2 therapy have a unique phenotype, in that a high proportion of them are also expressing CD25, which is usually expressed on activated memory cells. These cells appear to be different from the recently described $CD4^+$/$CD25^+$ suppressor cells that appear to play a role in preventing organ-specific autoimmune disease, in that the latter cells are primarily of the memory phenotype [16].

Like other lymphocytes, NK cells decreased during IL-2 therapy and then increased immediately following discontinuation of IL-2; however, they did not show any long-term increase in number in this or prior studies using intermittent administration of IL-2. This contrasts to studies in which lower doses of IL-2 are given subcutaneously on a daily basis for many weeks [6, 7]. In the latter studies, there is a significant increase in NK cell number that is maintained while IL-2 is administered, although $CD4^+$ cell numbers remain largely unaffected. Since intermittent subcutaneously administered IL-2 has immunological effects similar to intermittent intravenous IL-2 [4], it appears that the primary factor in determining which cell population increases is whether therapy is continuous or intermittent. Based on the available data, therapeutic strategies targeting increases in NK cells should utilize daily administration of IL-2, while strategies targeting $CD4^+$ cell number increases should utilize intermittent regimens.

This study has provided further insights into the complex cascade of events that are initiated by the administration of IL-2 to HIV-infected patients. Further characterization of these events should help our understanding of the mechanism of action of IL-2 both in HIV-infected patients and in other immunodeficient populations, and may lead to interventions that decrease IL-2-related toxicities or supplement efficacy. Studies addressing these issues, which will help define the role of IL-2 in the management of HIV infection, are currently underway.

4 Materials and methods

4.1 Patients

The primary study enrolled 11 patients with HIV infection and $CD4^+$ counts above 200 cells/mm^3 who had never received IL-2, had no history of a prior AIDS-defining opportunistic infection, and had received no corticosteroids, cytotoxic chemotherapy, or experimental therapy in the prior 4 weeks. The study was designed to obtain frequent measurements of immunological and virological parameters in the context of repeat 5-day infusions of IL-2. Virological and limited immunological changes seen in this cohort are being reported separately [8]. Fifteen patients meeting the same eligibility criteria were enrolled in an earlier phase of the study designed to obtain immunological information during a 21-day infusion of IL-2 at four dose levels; tolerability of IL-2 by this group has been briefly reported previously [2]. All studies were approved by the NIAID institutional review board, and all patients provided written informed consent.

4.2 IL-2 and anti-retroviral drug administration

IL-2 (Chiron Corp. Emeryville, CA) was diluted in 5% dextrose in water containing 0.1% albumin and administered as previously described by continuous infusion at a starting dose of 18×10^6 IU/d for 5 days, with dose reductions of 3×10^6–6×10^6 IU/day as needed for clinical or laboratory toxicities, which were similar to those reported in prior studies [2–5, 8]. The average dose received during the first year of the study was 46×10^6 IU per 5-day cycle. IL-2 cycles were administered approximately every other month for 1 year.

All patients received approved anti-retroviral drugs, which could be changed during the course of the study. The specific regimen was determined by the patient and referring physician with input from the study team.

4.3 Assessment

Immune parameters were evaluated daily for 10 days after beginning each IL-2 cycle, and at monthly follow-up visits. Determination of lymphocyte subsets and surface markers was performed as previously described [13, 17]. For distinguishing naive and memory cells, cells were stained with anti-CD45RO; memory cells were defined as RO positive, and naive as RO negative. In examining CD25 expression by naive and memory cells, antibodies to CD45RA and CD62L (fresh cells) or CD45RO and CD27 (frozen cells) were used to identify naive cells [18, 19]. Spontaneous blast transformation was performed as previously described [20]. Cell cycle determinations were performed using the Cellular DNA Flow Cytometric Analysis Reagent Set (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions, and analyzed using the Multicycle AV program (Phoenix Flow Systems, San Diego, CA). *Ex vivo* labeling of lymphocytes with BrdU (Sigma, St. Louis, MO) was used to examine the effects of IL-2 on the proliferation of lymphocyte subsets. Samples were obtained immediately prior to and on day 4–6 after starting IL-2 from a cohort of patients receiving 5-day cycles of intravenous or subcutaneous IL-2 as part of ongoing IL-2 trials. Whole blood was incubated for 4 h at 37°C with 10 μM BrdU, following which cells were processed for flow cytometry using antibodies to CD3, CD4, CD8, and BrdU as previously described [21].

4.4 Statistics

Pre- and post-IL-2 values were compared using a paired, two sided Student's *t*-test (SPSS version 6.1 for the Macintosh, SPSS, Inc. Chicago, IL). *p* values (two-sided) less than 0.05 were considered significant.

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